

Frog Deformities Research Not Leaping to Conclusions

Since 1989, evidence has been mounting of a global decline in the numbers of certain species of amphibians. Three years ago, biologists in Minnesota discovered a different problem in the amphibian community—large numbers of frogs with missing or deformed limbs. Subsequent research indicates the problem is widespread, including large areas of Minnesota, Wisconsin, New York, Vermont, and parts of Ontario and Quebec. Determined to track down the cause of these deformities, the Minnesota Pollution Control Agency (MPCA), based in St. Paul, has called on the NIEHS to collaborate on a research effort.

The first hint of trouble among frogs came in 1993, when a group of schoolchildren discovered frogs with malformed and missing limbs in a Minnesota farm pond, and posted their findings on the Internet. During the following two years, reports of deformities increased but were restricted to a few localities and one species, the northern leopard frog (*Rana pipiens*). In 1996, the number of reports and species

laboratory-reared frogs—less than 0.05%. Thus, biologists have little doubt they are witnessing a genuine phenomenon.

Starting in late 1996, workshops were held by various government agencies to postulate mechanisms that could result in the deformities, review possible environmental causes, and identify research needs. Participants agreed that a national report-

Scientists have advanced a number of theories to explain the deformities. Prime suspects include chemical contamination, increased ultraviolet (UV) radiation due to ozone depletion, parasitic infestation, or some combination of the three. Frogs spend a major portion of their lives in water, and thus may be particularly vulnerable to ill effects stemming from chemical

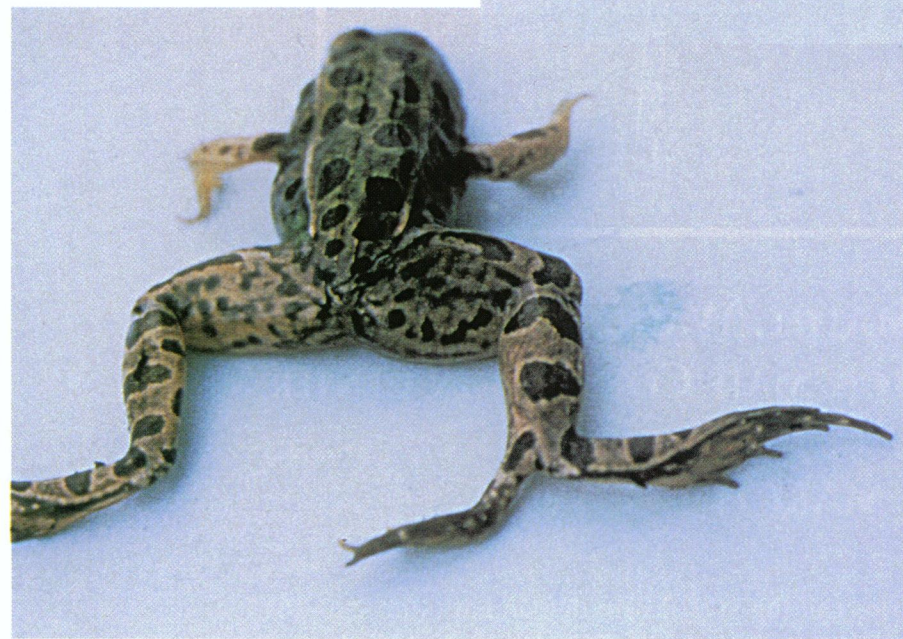


Unhappy frogs. Extra limbs are some of the deformities showing up in frogs in Minnesota, the cause of which is still unknown.

contamination of water. Many of the sites in which deformed frogs have been discovered are close to agricultural fields that are intensively sprayed with pesticides and herbicides at certain times of the year. It has been suggested that one or more of the chemicals used, acting alone or in combination with other agents such as UV light, might be disrupting normal frog development.

"There are several prominent cases in this country in which chemical agents were known to cause gross anatomical deformities in wildlife," says Tim Kubiak, national water quality coordinator for the U.S. Fish and Wildlife Service. "In the western United States, high levels of selenium leaching into surface water caused defects in the eggs of shorebirds and fish. And in the Great Lakes, dioxin and dioxin-related chemicals are suspected of causing crossed bills among fish-eating birds. So there is certainly reason to explore a chemical connection with the frog deformities."

Stanley Sessions, a biology professor at Hartwick College in Oneonta, New York, has argued that a class of parasitic flatworms known as trematodes may be



involved increased tremendously. Six species of deformed frogs have been documented by the MPCA at more than 150 sites across 54 counties in Minnesota. The malformations found include missing or additional feet, legs, toes, and eyes, as well as musculoskeletal and urogenital defects. The incidence of abnormalities was as high as 60% among some frog populations. Abnormalities are rare among natural and

ing center was needed to encourage standardization of the data being collected by federal, state, and university research groups. The National Reporting Center for Amphibian Malformities (NARCAM) has subsequently been established at the Northern Prairie Wildlife Research Center in Jamestown, North Dakota, which has an Internet site located at <http://www.npsc.nbs.gov/narcam>.

responsible for the deformities. Trematodes living in aquatic environments can induce deformities by boring into the skin of larval amphibians and causing cysts near the developing hind limbs. These cysts are hypothesized to disrupt normal development. However, parasitology information indicates that amphibians and parasites have long coexisted in equilibrium and that parasite populations tend to be very stable. Also, in recent testing, water samples from several sites where deformed frogs have been found from which all parasites had been removed still produced abnormalities in frogs.

As of yet, there are insufficient data to establish any correlation between chemical contamination, or any other cause, and the incidence of abnormality. In the spring of 1997, the MPCA requested help from the NIEHS to establish a collaborative research effort to tease out associations between chemical exposures and malformations, to demonstrate similar effects under controlled laboratory experiments, and to identify the mechanisms by which the deformities are occurring. Jim Burkhart, head of the Alternative Systems Group within the NIEHS's Environmental Toxicology Program, is coordinating this effort. Researchers from the EPA, the National Wildlife Health Center in Madison, Wisconsin, the U.S. Geological Survey's Environmental and Contaminants Research Center in Columbia, Missouri, and the University of Minnesota are also participating.

"We [the NIEHS] have a very broad base of scientific expertise that can be brought to bear on this issue, including environmental chemistry, different laboratory models, retinoid receptor models, and endocrine disrupter models," Burkhart says. "Our role is to determine cause and effect, and any possible relevance to human health."

The possibility that whatever is causing deformities in frogs may also be linked to human health is indicated by a 1996 study by University of Minnesota researchers (*EHP* 104[4]:394-399). The study showed an increased incidence of abnormality and chromosomal aberration among children born to pesticide applicators

in Minnesota. In western Minnesota, a major wheat, sugar beet, and potato growing region, the increase was particularly pronounced for children conceived in the spring, the time of heaviest pesticide application.

Field work in the MPCA/NIEHS collaboration began during the frog breeding season in July 1997. On 30 September 1997, the MPCA and the NIEHS held a joint press conference to convey results of preliminary studies. Water and sediment samples were collected from affected and unaffected ponds in Minnesota and tested using the Frog Embryo Teratogenesis Assay: *Xenopus*, known as FETAX. The assay involves exposing developing embryos of *Xenopus laevis* to toxins for four days and observing any resulting malformations. Although this is not the same frog species as those observed with naturally occurring malformations in Minnesota, at concentrations above 50%, water from the ponds with a high incidence of malformed frogs induced a high rate of malformations in the FETAX as well. There is good agreement for birth defects between the FETAX and rodent bioassays, which are often used as the basis for regulatory decisions concerning environmental contaminants and human health. Water from ponds that did not contain malformed frogs did not induce effects in the FETAX. However, preliminary findings suggest that similar effects are induced by groundwater and well water collected at residences near the affected ponds, prompting further concern for human health. The residents have been advised of the findings and offered the option of having the state provide bottled water as a precaution until more is known. George Lucier, director of the Environmental Toxicology Program at the NIEHS, says, "We know that something in the water, including groundwater, is extraordinarily potent in malforming frogs. We now need to determine if people are at risk."



Jim Burkhart

The goals of the MPCA/NIEHS project call for establishing continuity in the field observations taken over the past three years, and characterizing the frequency and nature of the malformations in frog larval stages and metamorphs. The researchers will continue to conduct chemical analyses of water and sediment from test sites where deformed animals have previously been observed, as well as from sites containing no deformed frogs, and attempt to determine the identity of the causative agent or agents. An approach to studying the affected waters in standardized rodent teratogenicity assays is being considered, and an evaluation of human health effects associated with living in the affected areas is planned in collaboration with state and federal epidemiologists. Says Burkhart, "The exciting thing about this is that, within Minnesota, we have a set of circumstances that provides a basis for study. With numerous sites containing confirmed deformities, we have at least some opportunity to determine cause and effect."

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Amphibian Malformities (NARCAM) on the web

<http://www.npsc.nbs.gov/narcam>